Effect of acidic and osmotic stresses on survival of Listeria monocytogenes through an in vitro model of the gastrointestinal tract

Johansen, Pernille; Larsen, Nadja; Siegumfeldt, Henrik; Arneborg, Nils; Jespersen, Lene

Publication date:
2014

Document version
Early version, also known as pre-print

FACULTY OF SCIENCE
UNIVERSITY OF COPENHAGEN

Effect of acidic and osmotic stresses on survival of *Listeria monocytogenes* through an *in vitro* model of the gastrointestinal tract

Pernille Johansen, Nadja Larsen, Henrik Siegumfeldt, Nils Arneborg, Lene Jespersen

Department of Food Science, Food Microbiology, University of Copenhagen, Rolighedsvej 30, 1958 Frederiksberg C, Denmark.

Abstract

Ingestion of food products contaminated with *Listeria monocytogenes* is one of the major routes for exposure to this human pathogen. Survival through the gastrointestinal (GI) tract is a critical factor for establishment of listerial infections. *L. monocytogenes* can survive and grow over a wide range of environmental conditions, such as low temperatures, high NaCl and low pH. Unpublished results have shown that survival and virulence gene expression in *L. monocytogenes* grown on cheese was affected by NaCl concentration and low pH.

The aim of this study was to investigate the vitality of *L. monocytogenes* single cells during passage through an *in vitro* model of the GI tract.

Materials and methods

Strains

The study was conducted on three *L. monocytogenes* strains characterized by different tolerance to NaCl stress; 15675 (less NaCl-sensitive) and strain 51779 (more NaCl-sensitive) (unpublished results), as well as the reference strain EGDe.

Stain procedure

Vitality of *L. monocytogenes* single cells were determined by fluorescent ratio-imaging microscopy (FRIM), using the pH-sensitive ratiometric probe 5(6)-carboxy-2',7'-dichlorofluorescein diacetate succinimidyl ester (CDCFDA-se). CDCFDA-se labeled cells were excited with 488 nm (pH-sensitive excitation wavelength) and 435 nm (pH-insensitive excitation wavelength) and emission intentisities from single cells were recorded. The ratio-value between the intensity (I) of the emitted fluorescence at 488 nm and 435 nm, was determined for each cell examined by the equation: $R_{488/435nm} = (I_{488} - I_{488\ background}) / (I_{435} - I_{435\ background})$.

In vitro GI tract model

One cover well chamber, mounted with a well for incoming solution and another for outgoing solution, were glued to a bind silane coated microscope glass slide.100 µL CDCFDA-se stained cells were pipetted to the chamber and centrifuged at 1000 ×g for 1 min to allow the cells to settle and attach to the bind silane. An inlet- and outlet high-precision peristaltic pump were used to create a continuous flow in the perfusion chamber over the stained cells. At time 0 cells were flushed with BHI, 1% NaCl, pH 7.0. In the first 5 min of passage in the *in vitro* model of the GI tract, cells were flushed with synthetic saliva (pH 6.5). Throughout the rest of the experiment (5 min to 110 min), cells were flushed with a mixture of synthetic saliva and gastric juice (pH 3.5), mixed according to Ramos *et al.* 2014. During passage in the *in vitro* model of the GI tract, emission intensities were recorded for single cells at start, after 5 min and here after every 15 min. The number of analyzed cells was; a) 15675 (n=19), b) 51779 (n=18) and c) EGDe (n=26).

Evaluation of ratio-curves

Linear regression slopes for ratio-values between two time points were calculated, as well as standard deviations for the obtained ratio-values at each time point for the three strains.

Table 1. Intra-strain variation in cell responses. Based on standard deviations for ratio-values of the analyzed cells.

Solution	Strain Time (min)	15675 sd	51779 sd	EGDe sd
BHI	0	0.598	0.384	nd
Saliva	5	0.398	0.285	0.307
Saliva + gastric juice	20	0.444	0.172	0.681
Saliva + gastric juice	35	0.645	0.189	0.468
Saliva + gastric juice	50	0.885	0.125	0.321
Saliva + gastric juice	65	0.996	0.188	0.213
Saliva + gastric juice	80	0.913	0.161	0.185
Saliva + gastric juice	95	0.817	0.157	0.198
Saliva + gastric juice	110	0.778	0.146	0.220

sd: standard deviation
nd: not determined

Results

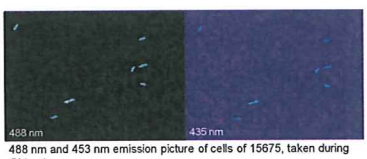
Figure 1, depicted the ratio-values of single cells for the three strains, measured during passage in the *in vitro* model of the GI tract. Cells of the more NaCl-sensitive strain, 51779, had the most pronounced drop in ratio-values within the first 5 min of the experiment, when exposed to synthetic saliva (pH 6.5), with an average ratio slope value of -0.38 between two time points. On the contrary, cells of the less NaCl-sensitive strain, 15675, showed overall different initial responses to synthetic saliva, resulting in average ratio slopes between 0.20 and -0.24. After changing the flush solution to a mix of synthetic saliva and gastric juice (from 5 min), the highest drop in ratio-values was observed for cells of EGDe, with average ratio slopes between two time points at -0.06.

Table 1, showed the intra-strain variation in cell responses during GI tract passage, based on standard deviations of the ratio-values. Cells of the less NaCl-sensitive strain, 15675, exhibited the highest intra-strain variation in cell responses at all included time-points, with standard deviations of ratio-values ranging from 0.398 to 0.996. Cells of the more NaCl-sensitive strain, 51779, had the lowest intra-strain variation in cell responses, where the ratio-value standard deviations were between 0.146 and 0.384. In between these two, was the intra-strain variation of cells of EGDe, resulting in standard deviations of ratio-values of 0.185 to 0.681. This indicated that the cells analyzed from the less NaCl-sensitive strain 15675 showed a higher degree of heterogeneity as an initial cell response to exposure to both synthetic saliva and the mixture of synthetic saliva and gastric juice, as compared to the initial cell responses of EGDe and 51779.

Conclusions

The synthetic GI tract solutions induced different initial cell responses in the three studied strains, as observed by differences in average ratio slopes, during GI tract passage.

During GI tract passage, the degree of heterogeneity in initial cell responses within the studied strains varied, as seen by differences in intra-strain variations.



488 nm and 453 nm emission picture of cells of 15675, taken during GI tract passage

Epiuorescence microscope (Axiovert 135 TV; Zeiss, Birkerød, Denmark)

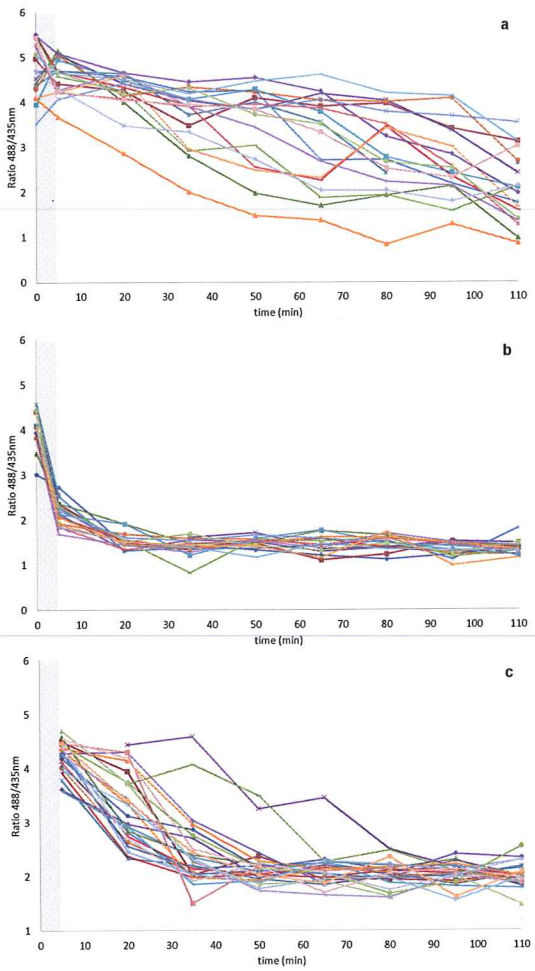


Figure 1. *In vitro* GI tract passage of single cells of; a) 15675, b) 51779 and c) EGDe

Graphs representing ratio-values of emission intensities from excitations of CDCFDA-se labeled cells with 488nm (pH-sensitive excitation wavelength) and 435 nm (pH-insensitive excitation wavelength). Cells were added to a flow chamber and flushed with synthetic saliva solution for 5 min (pH 6.5), marked by the grey area on the graphs. Subsequently, cells were flushed with synthetic gastric solution (pH 3.5) for 105 min, prepared according to Ramos *et al.* 2014. Single cell emission intensities were recorded throughout the experiment, representing the cell responses of a) 19 15675 cells, b) 18 51779 cells and c) 26 EGDe cells. Emission intensities for c) 0-5min were not recorded. Prior to addition to the flow chamber, the three strains were grown to exponential cultures (OD_{600} = 0.2-0.3) in BHI (pH 7.0) at 20°C.

Acknowledgements

The work was supported by the project: "New Strategies for estimation of virulence potential of foodborne pathogens by quantitative gene analyses" (GeneQuant)

Funded by:
The Danish Agency for Science, Technology and Innovation, Denmark
The Danish Dairy Research Foundation
The Ministry of Food, Agriculture and Fisheries, Denmark

Cited references

Ramos, C. L., Thorsen, L., Ryssel, M., Nielsen, D. S., Siegumfeldt, H., Schwan, R. F., & Jespersen, L. (2014). Effect of the gastrointestinal environment on pH homeostasis of *Lactobacillus plantarum* and *Lactobacillus brevis* cells as measured by real-time fluorescence ratio-imaging microscopy. *Research in Microbiology*, 165(3), 215–25.